Medical Science

25(115), September, 2021

Development of nano-formulating dexamethasone for anterior uveitis treatment: A randomized clinical trial study

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To Cite:
Feghhi M, Makhmalzadeh BS, Farrahi F, Sharafi A, Kasiri A. Development of nano-formulating dexamethasone for anterior uveitis treatment: A randomized clinical trial study. Medical Science, 2021, 25(115), 2440-2451

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Peer-Review History
Received: 21 August 2021
Reviewed & Revised: 22/August/2021 to 16/September/2021
Accepted: 18 September 2021
Published: September 2021

Peer-review Method
External peer-review was done through double-blind method.

ABSTRACT

Objective: To evaluate the efficiency of nano-formulating dexamethasone in the treatment of anterior uveitis. *Methods:* The present study is a randomized clinical trial study conducted on 20 patients with anterior uveitis referred to the Ophthalmology Department of Imam Khomeini Hospital, Ahvaz University of Medical Science from June 2019 to March 2020. Patients were divided into two groups randomly with 10 subjects in each group. One group of patients received micellar nanoparticle containing 0.1% dexamethasone (DEX) (the nanoparticle group) and another group received 0.1% DEX solution (the control group). In the 1st, 3rd, 7th, 14th, and 28th day of the treatment and second month after treatment, the patients were followed-up clinically. The number of visible cells in the anterior chamber are counted and graded on a scale of 0 to 4 based on the Standardization of Uveitis Nomenclature Working Group. Visual acuity, posterior adhesion, cell count of anterior chamber, flare, hypopyon, and intraocular pressure was compared between the two groups. Besides, the correlation between hypopyon and vision, the cell count in the anterior chamber and visual acuity, visual acuity and flare, visual acuity and posterior adhesion. *Results:* There was a difference in visual acuity, removal of posterior adhesion, cell count of anterior chamber, flare, improving hypopyon, intraocular pressure significantly between the nano DEX group and the control group ($P<0.05$). *Conclusions:* The use of 0.1% DEX nanoparticles can be quite effective in anterior uveitis treatment. Therefore, more controlled studies are needed to understand and recognize the effects of DEX nanoparticles in the treatment of uveitis.

Keywords: Uveitis; Nano-micellar compounds containing dexamethasone; Flare; Hypopyon

1. INTRODUCTION

The uvea is composed of avascular and intermediate structures of the eye, which includes the iris, ciliary body, and choroid. Uveitis is an autoimmune disease and inflammation of the uvea system that can affect other parts of the eye (Jaffe et al., 2016; Chee & Khairallah, 2017). The classification of uveitis is

based on the anatomical location of the inflammation and the clinical and etiological course, so that the uveitis in terms of the anatomical location of the intraocular inflammation is divided into anterior uveitis (cyclitis, iritis and iridocyclitis), intermediate uveitis (pars planitis), posterior uveitis (diffuse choroiditis, chorioretinitis, focal or multifocal retinitis and neuroretinitis) or panuveitis (Markomichelakis, 2017).

Uveitis is blamed for 10% of blindness and each year approximately 30,000 new cases diagnosed in the United States (Crowell & Reddy, 2017). The anterior uveitis usually occurs among the ages of 20 and 50 years old, and may involve one or both eyes. Treatment of posterior and panuveitis is challenging because the anatomical and physiological properties that effectively protect the eye in these areas, decreasing drug absorption. Immunosuppressive drugs and corticosteroids have beneficial effects in the treatment of uveitis (Kulkarni, 2001). Mydriatic and cycloplegic drugs are also prescribed topically to prevent posterior adhesions and reduce secondary photophobia to ciliary spasm in uveitis patients (Islam & Pavesio, 2015). Nevertheless, delivery of these drugs into the retina is challenging, and ophthalmologists sometimes have no choice except topical inject through intraocular or subcutaneous injections and implants, which are both highly invasive and have several known side-effect (Duvvuri et al., 2003). Although intraocular injections of these drugs are boasted for the higher concentration in the eye, the risk of drug overload (especially for lipophilic drugs), short half-life of the drug, and patient incompatibility can reduce the effectiveness of this therapeutic approach (Beer et al., 2003). In addition, chronic uveitis requires multiple injections, which increases the risk of vitreous hemorrhage, retinal detachment, and cataract progression (Shah et al., 2018).

Implantation (such as Retisert fluocinolone acetonide intravitreal implant) eliminates many of the disadvantages of intraocular injection, however, the surgical procedure and risk of loading and deposition may lead to adverse effects (Haghjou et al., 2011). The Food and Drug Administration has confirmed the intraocular injection of dexamethasone (DEX) intravitreal implant (Ozurdex®) for the treatment of posterior uveitis, but it has many side-effect such as high intraocular pressure, vitreous hemorrhage, itching, implant migration to the anterior parts of the eye and corneal destruction (Hunter & Lobo, 2011). Topical eye drops can reduce the side-effect associated with intraocular implants and intraocular injections, hence improving the patient's quality of life (Patel et al., 2013). Although corticosteroids are used as a first-line treatment for uveitis patients, they have many side-effects, low bioavailability, and low permeability to different parts of the eye, such as the retina (Babu & Mahendradas, 2013).

Delivery of drugs dose to the eye is a serious challenge particularly for the treatment of inflammation of posterior segment of the eye (Tombran-Tink & Barnstable, 2007). Therefore, various strategies are developed for improving ocular drug bioavailability and increase the permeability of drugs to corneal. One of these strategies is the use of nanotechnology-based drug carriers that have been widely studied over the past few decades (Weng et al., 2017). Polymeric micelles are colloidal particles with a size around 10-100 nm. Polymeric micelles consist of amphiphilic block polymers and characterized by a core-shell type of architecture, where the inner core is hydrophobic and the outer shell is hydrophilic by nature (Hanafy et al., 2018). Micelles can enhance the aqueous solubility of hydrophobic/lipophilic drugs. Polymeric micelles have currently attracted attention because of their capability of solubilizing the poor soluble drugs, higher ocular bioavailability, stronger efficiency with less dose, and rareside-effect (Lu & Park, 2013). These compounds can bind to cellular barriers and ameliorate penetration of drugs *via* the cornea because that they have a similar size range as membrane proteins, scleral pores (cornea), and other biomolecules (Lu et al., 2018).

Features such as less toxicity, increased drug stability in the core, easy preparation method, relatively simple sterile production, either spatial or temporal control of drug delivery, not interfering with the vision of patient due to its aqueous character and constant drug release occurs as the core acts as a drug depot. Nanoparticles reduce the number of drug doses, as an effective treatment system to deliver the drug to the desired area (Mandal et al., 2017). The small size of these compounds leads to increased permeability and retention so that they can reach target inflamed regions. On the other hand, the drug trapped in nanoparticles acts as a drug reservoir on the surface of the cornea, reducing the rate that the drug is discharged from the eye (Pepic et al., 2012). Also, in the design of polymer micellar compounds, heat-sensitive factors have been used, which can be formed by placing a gel structure at body temperature, which increases the shelf life of the drug at the target site (Kulthe et al., 2012).

Besides, the compound used has a positive electric charge, which increases the durability of the drug on the surface of the cornea by connecting to the negative electric charge of the eye surface and by the effect of mucosal adhesion. Due to the low bioavailability of corticosteroids used in the treatment of uveitis, followed by the problem of drug delivery into the eye due to the dynamic anatomy and the blood-ocular barriers (including blood-aqueous and blood-retinal barrier), Irimia et al., (2018) used polymer micellar compounds to solve this problem. Therefore, in this study, in order to increase the penetration of the drug into the eye and reduce the side-effect of the drug in the eye, we decided to use micellar nanoparticle containing 0.1% DEX to treat uveitis patients and evaluate the therapeutic effect of this drug on uveitis patients.

2. SUBJECTS AND METHODS

Synthesis of di-block polymer

The di-block polymer was synthesized by ring opening polymerization with necessary modifications. Briefly, initiator mPEG (3 mmol), catalyst stannous octoate (0.5% w/w of reactants) and monomer ε-caprolatone (39.4 mmol) were added in a response vessel prefilled with nitrogen. Toluene (5 mL) was added to the reactants, surveyed by heating at 100℃ under vacuum. The reaction mixture was dissolved in dichloromethane and occasioned in cold anhydrous ether. The final product was filtered and dried under vacuum

Critical micelle concentration (CMC)

CMC was determined using pyrene as a hydrophobic fluorescent probe following a previously published method with modifications (Lu et al., 2018). Deuterium-depleted water (DDW) was added to the dried samples and vortexed for 1 min. Solutions were incubated at 37℃ for 12 h then removed undissolved pyrene by syringe-filtered (0.25 μm). Filtrates were measured for pyrene fluorescence. Samples were excited at 330 nm, and emissions were measured at 372 nm (I2) and 392 nm (I1). A ratio of emission intensities (I2/I1) was plotted against polymer concentrations to calculate CMC. The polymer concentration where we observed a sharp increase in I2/I1 ratio was considered as CMC for the polymer.

Preparation of DEX-loaded micelles

Thin-film hydration method was used for preparation DEX-encapsulated micelles. Briefly, measured amounts of the DEX (10 mg) and PCL-PEG-PCL copolymer (200 mg) were thawed in 2 mL of tetrahydrofuran (THF). After organic solvent was totally vanished to generate matrix films, 1 mL of deionized water was added to each film and stirred incessantly until it was fully dispersed. After ispersions were heated to 60℃ until the copolymer was melted, quenching done in an ice bath for one minute (Billerica, Millipore™) to isolated undissolved drug and accumulated polymers and were then assessed for DEX solubility by reverse phase high-performance liquid chromatography. The loaded drug was released by diluting a certain volume of preparation in THF, and the amount of DEX in the dilute solution was determined using a calibration curve of DEX in the THF/acetonitrile mixture.

Study population, grouping and randomization

This study is a double-blind randomized clinical trial study conducted on patients with anterior uveitis referred to the Ophthalmology Department of Imam Khomeini Hospital, Ahvaz University of Medical Science from June 2019 to March 2020. Patients with recurrent uveitis, chronic vision-threatening uveitis, previous intraocular surgery, and retinal problems were excluded from the present study. The included patients were divided into two groups randomly with 10 patients in each group. The selection of patients in both groups was completely random and before starting the treatment, the necessary explanations about the treatment process and how to follow the patients were given by physician and the informed consent was obtained and recorded before entering the study. The randomization method was based on the block chain method, and the individuals were divided into two groups randomly (by a person who was not involved in the study). For this purpose, six blocks of AABB, ABAB, ABBA, BAAB, BBAA and BABA were considered, which were sampled with the number of N/4s and placed with them. Patients and the interventionist and the individual who reviewed the results were unaware of the group of individuals, and the study was conducted double-blinded.

Intervention

One group of patients received micellar nanoparticle containing 0.1% DEX (the nanoparticle group) and another group received 0.1% DEX (the control group). All of the cases in both groups had acute non-infectious single-eyed uveitis. Because of the possibility of intraocular pressure rising due to both nanoparticles and conventional drugs, first intraocular pressure was measured in each patient and patients with high intraocular pressure were excluded from further study. All patients in the two groups were examined by a rheumatologist and an internal medicine physician to comfirm non-ocular complications related to immune system diseases. If any of the patients are candidates for systemic corticosteroids or immunosuppressive drugs with the opinion of a rheumatologist, they should be excluded from the study. Each of the patients who had intraocular and extraocular reasons affecting the process of vision and reducing or increasing the severity of inflammatory eye disease was prevented from entering the study and only patients with fixed and acute anterior uveitis were entered in the study groups (Figure 1).

The control group received the topical DEX 0.1% drop at the beginning of treatment every 4 h and the nanoparticle group received micellar nanoparticle suspension containing 0.1% DEX (manufactured by Sina darou laboratories company with a

standard health license) with the same initial dose every 4 h. The drugs of the two groups were placed in similar packages and delivered to the patient by another colleague, and the patient was taught how to take the drug. At the time of referrals and follow-ups, the treating physician and the researcher were not aware of the nature of the drug used. According to the nature of the disease and the response to treatment, the dose was reduced during treatment and follow-up if necessary.

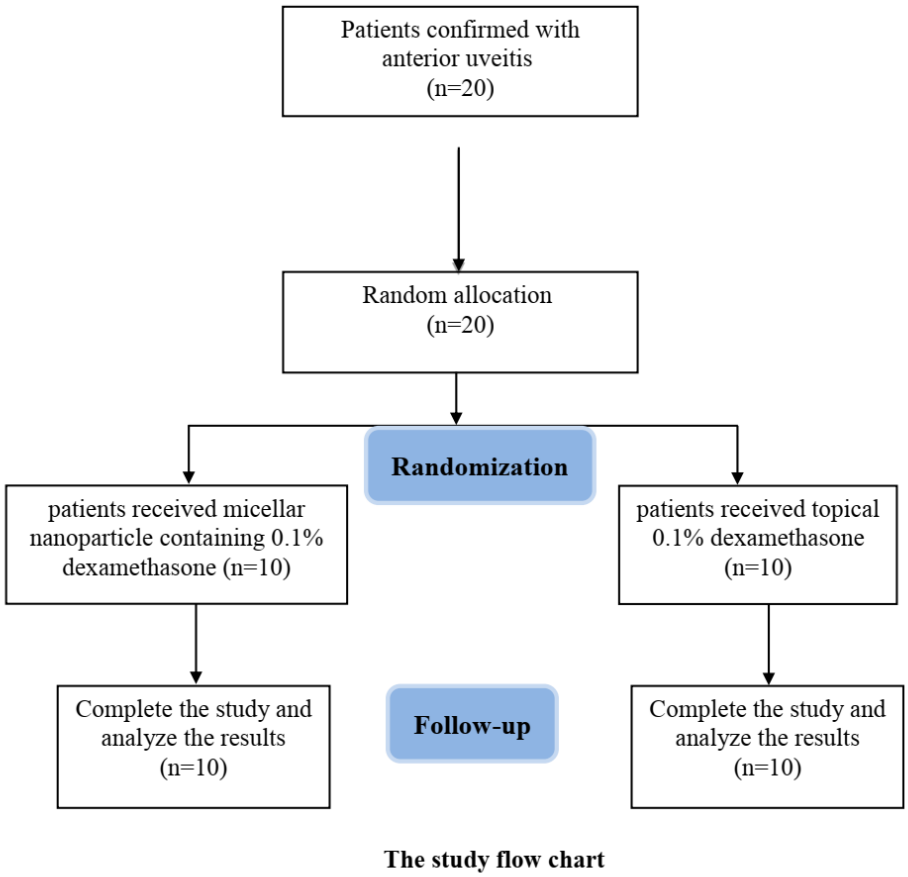

Figure 1 The study flowchart

Evaluating treatment

Dosage reduction in both groups was according to the cellular response of the anterior chamber, so that in both groups, according to anterior chamber cell 4 plus, one drop of the drug was poured every 4 h in the lower cul-de-sac and during follow-up as for the reduction in the number of cell count in the anterior chamber, the amount of drug also decreased. So that in the amount of cell 3 plus dose of the drug every 6 h, in cell 2 plus dose every 8 h, and cell 1 plus dose every 12 h was reduced. Finally, whenever the anterior chamber cell became zero, the dose was injected once a night until the next visit and in the second visit, when the cell anterior chamber was zero, the drug was discontinued. According to the definition, the presence of even a small amount of white blood cells in the anterior chamber can be considered as anterior uveitis. However, in order to better compare the two groups and equalization of patients in terms of anterior chamber cell count, patients with at least 4 plus white blood cells in the anterior chamber were entered in the study so that the amount and dose of drug prescribed in patients in both groups were the same once every 4 h.

Other treatments for both groups, such as topical cycloplegic every 8 h if necessary, and anticoagulant drugs were used in both groups. Then on the first and third days and the first, second and fourth week and the end of the second month results and clinical features of the study were categorized in both groups and compared with each other statistically. After prescribing the drug and in the first days and day 3, day 7, day 14, day 28, and the end of the second month, the patients were followed up clinically. The researcher's variables such as patients vision using a visual chart, cell count, flare value, value of hypopyon based on millimeters in the anterior chamber of the eye, and amount of posterior pupil adhesion to the anterior lens capsule based on the degree, age and sex of the patients, and in each examination, the amount of intraocular pressure based on millimeters of mercury with a Tonopen device was evaluated and entered into excel software.

Complications and other problems studied in patients including eye pain, photophobia, epiphora, complaints of decreased vision and other symptoms were compared based on rankings in the two groups. The standard system of uveitis classification, (sun) working group grading scheme for cellular response of the anterior chamber 0 to 4 positive with the help of biomicroscopy of slit lamp and for measuring intraocular pressure from Goldman tonometer device and according to millimeter of mercury 10 was considered. Inflammatory complications of the disease, such as edema of macula and epithelial membranes of the membranes and retinal ischemia, and the presence or absence of neovascularization of retina and choroid before and after treatment, were considered during follow-up. During treatment in both groups, in case of increased intraocular pressure, the patient was treated with anti-glaucoma drug and the dose was reduced.

Statistical analysis

Analysis of data was completed by version 22 of SPSS (SPSS Inc., Chicago, IL, USA). In present study, formula of unpaired two-sample *t*-test was used to apprize the effect of the drug on the eye and also to determine the significance of the difference among the mean effects of the drug. The significant level of this study was set at α=0.05.

3. RESULTS

Comparison of visual acuity in patients among the two groups

In this study, the mean age of patients in the nanoparticle group was 36 years and ranged from 19 years to 48 years. The mean age of patients in the control group was 34 years and ranged from 20 to 45 years. Besides, 6 males and 4 females composed of the nanoparticle group, while 5 males and 5 females of the control group. The Figure 2 shows that the visual acuity over time in the nanoparticle group was much better than the control group, in other words, the effectiveness of DEX nanoparticle drug is much better than topical 0.1% DEX. On average, in the third follow-up, which is the third day after the start of treatment, visual acceleration is observed in the nanoparticle group. In the control group, the process of accelerating the improvement of central vision is slower, and there is a significant jump in visual improvement in the fourth follow-up, which was the end of the first week of treatment. The average difference in visual acuity between the nanoparticle dexamethasone group and the control group in the first follow-up is less than one line, but in the final visit, this visual difference increases to about two lines, which is in favor of a better effect of nan-drug on the patients' vision.

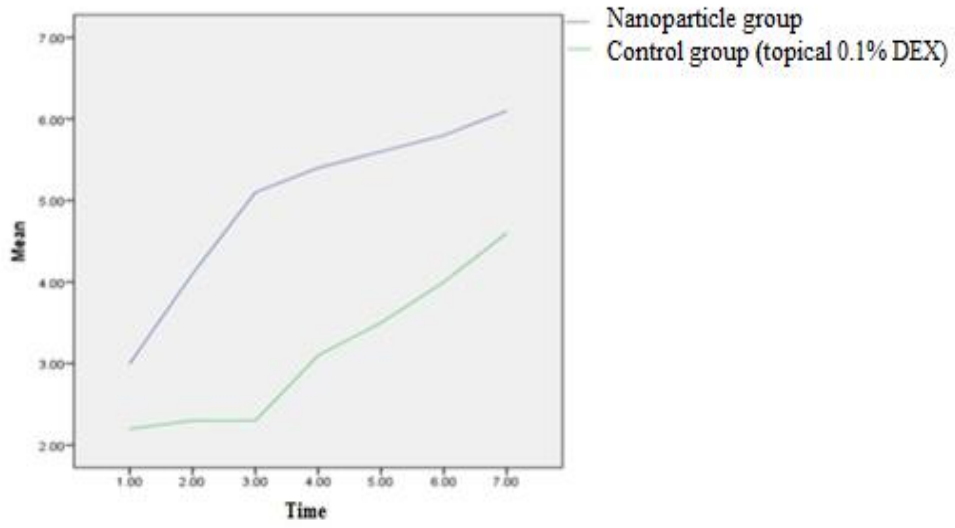

Figure 2 Comparison of vision variations in the two groups.

Comparison of posterior adhesion between the two groups

Figure 3 shows that the adhesion rate in the nanoparticle group reached zero at the period of the fourth follow-up, while this result occurred in the seventh follow-up for the control group. The blue graph is related to the amount of posterior adhesion of the nanoparticle group, which is initially with an average of 330 degrees (every 30 degrees of adhesion is considered equal to one unit). This trend in the control group initially with an average adhesion rate of 240 degrees (per unit in the chart is equivalent to 30 degrees of adhesion) reached zero in the fourth follow-up, which is the 7th day of treatment. The anterior lens capsule was

completely isolated in control patients, but this process occurs in the nanoparticle group on the third day. This difference is statistically significant (P<0.05). Patients in the nanoparticle group have their posterior adhesions removed sooner and the pupil separated from the lens capsule.

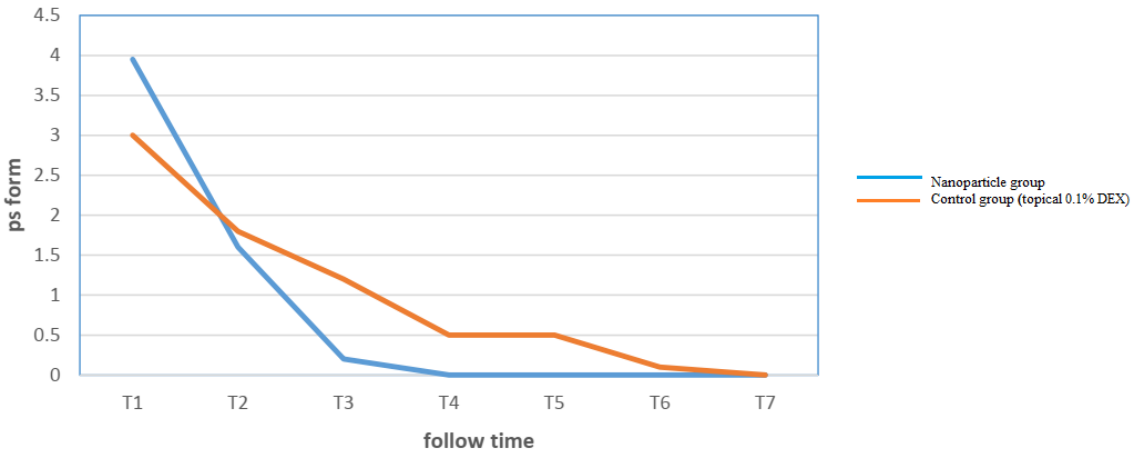

Figure 3 Comparison of posterior adhesion in two groups.

Comparison of cell count in two groups

The most important variable in the two groups is the cell count of the anterior chamber. The cell count of the anterior chamber is evaluated based on the SUN group criteria (Liu et al., 2019). In Figure 4, the blue line is related to the process of cell reduction in the nanoparticle group and the green line is related to the control group. Clearance time of the anterior chamber and zero cell count in the nanoparticle group occurred on average in patients at the period of the fourth follow-up, *i.e.* on the 7thday after the start of treatment, but in the control group, clear time of the white blood cell in the anterior chamber occurred in the seventh follow-up, meaning that in the control group, after one month, the anterior chamber becomes moderately clear in patients. The differences among the two groups was statistically significant (P<0.05) and with a high degree of confidence (95% *CI*:), indicating that nano-DEX drugs was more rapidly exposed to the clear, cell-free anterior chamber.

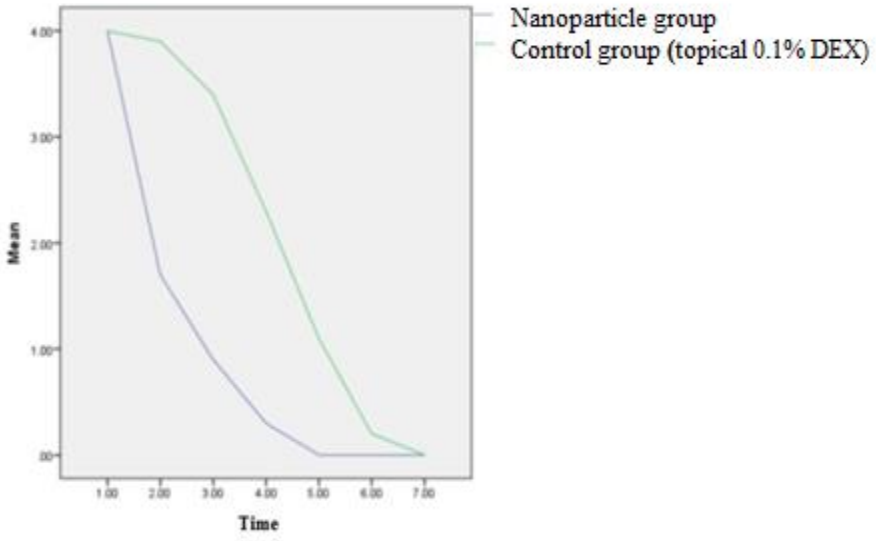

Figure 4 Comparison of cell count in the two groups.

Comparison of flare in the two groups

According to the definition of flare, it means the abnormal appearance of light beam reflected from the anterior chamber part of the eye, and the reason is the presence of abnormal proteins in the anterior chamber due to inflammatory disorders in the eye. A standardized international grading system for grading flare based on the SUN group criteria was applied (Sen et al., 2011). In

Figure 5, the blue line shows the decrease in the amount of flare in the nanoparticle group, and the green line is related to the decrease in the amount of flare in control group. The average amount of flare in the nanoparticle dexamethasone group and the control group is 2 plus and 1.5 plus, respectively. In the nanoparticle group, the time to zero of the anterior chamber intensity of the flare and the clearing of the eye is related to the 3rd follow-up on average. But in the control group, this decrease occurred in the 6th follow-up, meaning that at the 4 weeks of treatment. It is noteworthy that there was no significant difference on average on the third day of follow-up in the control group in term of the amount of the anterior chambers flare, while in the same period in the nanoparticle group, the average amount of flare was zero. The obvious difference among the two groups is significant in terms of flare, and this difference is statistically significant (P<0.05).

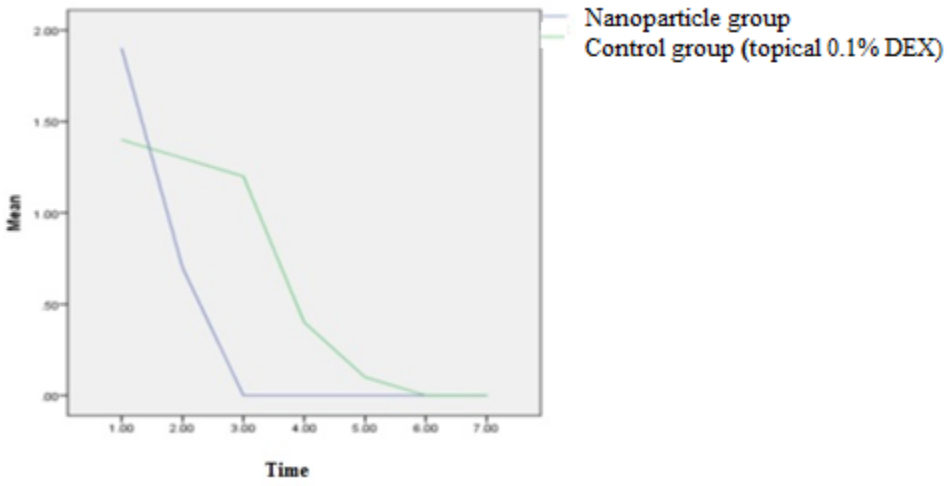

Figure 5 Comparison of flare in the two groups

Comparison of hypopyon in the two groups

Hypopyon is a less common clinical sign in patients with anterior uveitis and is the result of inflammatory cell accumulation in the anterior chamber. In Figure 6, the blue line is related to the decreasing trend of the amount of hypopyon in the nanoparticle group, and the green line is related to the control group. On average, hypopyon becomes zero in the nanoparticle group on the first day after micellar nanoparticle drug treatment, and this variable becomes zero in the control group during the fifth follow-up period, the second week. The statistical difference among the two groups is significant in terms of hypopyon (P<0.05), and in the nanoparticle group, hypopyon improves faster.

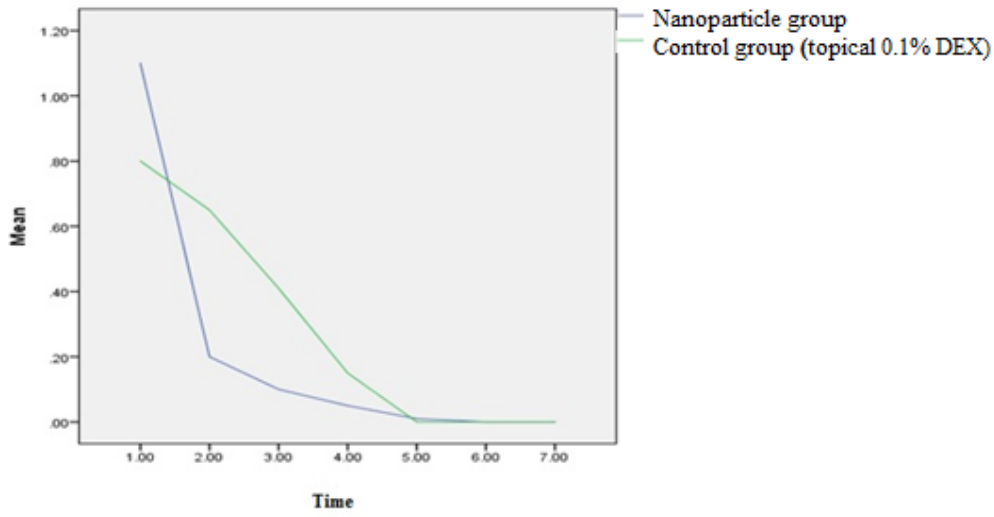

Figure 6 Comparison of hypopyon in two groups.

Comparison of the trend of intraocular pressure changes in the two groups

Intraocular pressure in both groups was assessed by Goldmann tonometer at the beginning of treatment and at intervals of the first day after treatment, day 3, day 7, day 14, and the end of the first month and the second month. The trend of intraocular pressure in the nanoparticle group was between 10 and 21 mm Hg for each patient in the normal range, and none of the patients in the nanoparticle group experienced a significant increase in intraocular pressure. The mean intraocular pressure in the nanoparticle group is unchanged, but in the control group at the second month's visit, about 2 mm Hg increased intra ocular pressure is observed in the total number of patients. Figure 7 shows that patients treated with nano-DEX typically show a slight increase in intraocular pressure over the course of 2 months, but this increase in pressure did not occur for each patient and did not require antiglaucoma therapy. Of the total number of patients in the control group, one patient experienced a slight increase in intraocular pressure after 8 weeks and with temporary administration of topical antiglaucoma (timolol), and then intraocular pressure returned to normal for a short time, and the drug was discontinued.

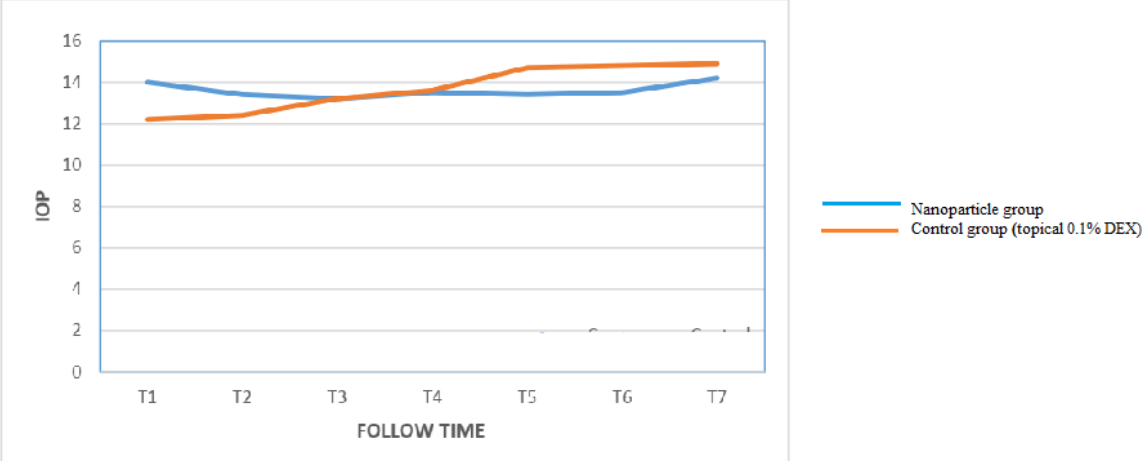

Figure 7 Comparison of the trend of intraocular pressure changes in the two groups.

Correlation between hypopyon and vision in the two groups

The results demonstrate that the reliability value is 0.05 in the nanoparticle group. Because it is equal to 0.05 (α=5%), at the 5% level, the correlation between hypopyon and vision in the nanoparticle group is significant. The value of correlation between the two relevant variables with a high reliability coefficient of -0.23 is negative, which shows that by reducing the amount of hypopyon, the best corrected vision of patients in this group increases, and this correlation is significant. The results show that because the reliability value is 0.001 in the control group. Because it is less than 0.01 (α=1%), at the 1% level, the correlation between hypopyon and vision in the control group receiving 0.1% DEX is significant. It has a correlation between the two variables of hypopyon and negative vision (-0.49590). This means that in the control group, there is a correlation between hypopion and visual acuity and with the decrease of hypopyon, the visual acuity of patients also improves.

Correlation between the anterior chamber cell count and visual acuity in both groups

The results show that with a very high degree of confidence and P>0.0004, there is a significant correlation between anterior chamber cell and patient visual acuity in the nanoparticle group, and the correlation coefficient of -0.41 means that the two variables are correlated, and with the decrease of the white blood cell in the anterior chamber of the eye, the amount of visual acuity also increases. The results show that there is a significant correlation between visual acuity and anterior chamber cell count in the control group with a very high confidence percentage and P> 0.0001 and this relationship is weaker in terms of correlation compared with the nanoparticle group (in the nanoparticle group, the correlation between visual acuity and cell is closer to 1).

Correlation between visual acuity and flare in the two groups

In both the nanoparticle and the control group, there is a significant correlation between visual acuity and flare with a high percentage of reliability. In the nanoparticle group, this correlation is closer to 1, which means that in the above groups, positive changes in the process of improving vision have occurred earlier by reducing flare.

Correlation between visual acuity and posterior adhesion in two treatment groups

Based on the correlation coefficient between the posterior adhesion variable with visual acuity in both groups, and given that the confidence interval in the nano-drug group is statistically significant (P<0.005), therefore, it can be concluded between the above two variables in the nanoparticle group that the visual acuity is correlated with the amount of posterior adhesion, but in the control group this event is not statistically significant (P<0.29).

4. DISCUSSION

Treatment of both anterior and posterior uveitis is a significant challenge. It is due to the anatomical and physiological protective properties of the eye, which reduce drug uptake (Agrahari et al., 2016). Immunosuppressive drugs and corticosteroids have valuable effects in the suppression of uveitis. Midratic and cycloplegic drugs are also given topically to uveitis patients to prevent posterior adhesions and reduce secondary photophobia to ciliary spasm (Kishore & Schaal, 2013). However, the delivery of these drugs and their passage through the cornea of the eye is a challenge in the treatment of uveitis, and ophthalmologists sometimes have no choice but to inject locally by intraocular injection or subcutaneously and implantation, which are very invasive and have many side effects. Although intraocular injection of these drugs increases the concentration of these drugs inside the eye, the risk of drug loading (especially for lipophobic drugs), short half-life of drugs and patient incompatibility reduce the effectiveness of this treatment (Sherman & Cafiero-Chin, 2019). The higher the lipophilic and hydrophilic potency of the drug or drug carrier, lead to the higher the amount and rate of drug passage through the cornea and the higher the pharmacodynamics of the drug (Jiang et al., 2018).

Posterior uveitis treatment needs delivery of corticosteroids to the choroid layer and retinal vessels (Sudharshan et al., 2010). FDA has approved the intraocular injection of DEX Ozurdex implant for the treatment of posterior uveitis, but this method has many side effects such as high intraocular pressure, vitreous hemorrhage, dry eye and implant migration to the anterior parts of the eye and Corneal destruction (Dugel et al., 2015). Topical eye drops can reduce the complications associated with intraocular implants and intraocular injections, thus improving the patient's quality of treatment (Sapino et al., 2019). Due to the low bioavailability of corticosteroids used in the treatment of uveitis and the problem in transmitting the drug into the eye, In this study, we used micellar polymer compounds (using Micellar nanotechnology and nanosuspension) containing corticosteroids (DEX) with a concentration of 0.1% to relieve intraocular inflammation for a group of patients as a case group. Therefore, in this study, in order to increase the penetration of the drug into the eye and reduce the side effects of the drug and the greater effect of the drug in the eye on the use of DEX nanoparticles for the treatment of uveitis patients and evaluated the therapeutic effects of this drug in anterior uveitis patients. We also used the control group, which received the usual DEX (Dexon 0.1%) as drops, to better compare the patient response process.

Based on the statistical results of this study group of anterior uveitis patients who received nano DEX treatment underwent treatment much faster than all the other criteria. The most important factor in terms of examinations was the level of central visual acuity, which occurred faster in the nanoparticle group. Another important variable was the amount of anterior chamber cells in patients. In patients in the nano-drug group, this variable underwent a faster recovery process and the anterior chamber was cleared between examinations on the seventh to fourteenth days in this group. However, in the control group, in the intervals of the sixth to seventh examinations, after about 4 weeks, the anterior chamber became clear. Other variables such as anterior chamber hypopyon and the amount of flare and posterior adhesion also improved faster in the nano DEX group and this difference among the two groups was statistically significant (p<0.05). In both groups, according to previous studies, we expected an increase in intraocular pressure in patients. Fortunately, in both groups, there was no significant increase in intraocular pressure and only one patient in the control group treated with normal DEX had an average increase in intraocular pressure in the second month which controlled and treated with an antiglaucoma drug and no increase in intraocular pressure was observed in subsequent follow-ups. None of the patients in this trial had recurrence of the disease until three months after the end of treatment and no specific side effects were occured in any of the treated patients in the two groups. In addition, drug sensitivity or drug intolerance and corneal and conjunctival complications were not observed in any disease.

Recently, due to the widespread use of nanotechnology in various fields and many studies on its ability, use this technique in the treatment of eye infections has been proposed. Better penetration of antibiotic particles into the eye can play a role in the faster healing process in some eye diseases. Previous experiments have revealed successful application of biodegradable polymeric systems in ocular and non-ocular environments (Tsai et al., 2018). Recently, the intraocular drug ciprofloxacin in the form of nanoparticles has been used in the treatment of bacterial endophthalmitis induced in rabbit eyes and has had satisfactory results (Feghhi et al., 2020). The reason for using nanoparticles is the permeability of these materials in different places in a programmed

manner that can be used for drug delivery. Since the number of studies using nanotechnology is not large, there is a need for more studies in the future (Xu et al., 2013).

In the present study, the permeability of conventional DEX and micellar DEX nanoparticles was compared. In all cases, the effectiveness of micellar nanoparticle containing 0.1% DEX was significantly higher than DEX 0.1% drop on the first day after treatment, which is similar to previous studies. Important limitation in comparing the results of different studies is the possible differences in methods and patient groups between studies. When comparing our visual results with other published reports, we conclude that DEX nanoparticle drops improve vision in patients with anterior uveitis, which is comparable to cases of invasive surgery or corticosteroid formulation, DEX implants, flucosinolone implants, and triamcinolone acetonide injections into the vitreous. All of these drugs have been used in the treatment of posterior eye inflammation and no human clinical trial using nanoparticles has been performed in anterior eye inflammation, but our study shows that the ratio of vision improvement in patients with edema of macula due to uveitis with Ozurdex is three lines, micellar DEX nanoparticles may be better than Ozurdex's (Medeiros et al., 2014).

In the Cohen et al., (1997) study in the animal models with endotoxin-induced uveitis (EIU) for assessment of the possible therapeutic efficacy, it was shown that topical administration of DEX failed to reduce inflammatory symptoms in uveitis. The scientists claimed that topical treatment with DEX was ineffective and that the therapeutic concentration of the dexamethasone in the anterior chamber did not reach the level required to inhibit uveitis (Tombran-Tink & Barnstable, 2007). However, previous studies such as the Mitra Alami-Milani study in 2018 have shown that the DEX compound synthesized in PCL-PEG-PCL micelles can be an effective drug combination and significantly improved patients' condition. This is due to its transcorneal penetration compared to DEX eye drops. Her next study, conducted in 2019 in rabbits with Salmonella endotoxin-induced uveitis, found significant differences in nanoparticle treatment but did not show a major difference with tropical DEX (Alami-Milani et al., 2019). Comparing our study with these studies, in recent studies the models were all animals, but the subjects were humans in our study, but the results of the better efficacy of nanoDEX in our study were more and the difference between these studies and our study was inflammation caused by Endotoxin factor.

In a study by Feghhi et al., (2020) the effect of nano-DEX compounds was compared to topical DEX interms of symptoms of inflammation including conjunctival changes, corneal edema, red eye reflex and cellular response of the anterior chamber of the eye. This study results showed that there was a major difference in the penetration rate of nano-DEX toward conventional DEX. Significant differences were also observed in terms of reducing inflammatory symptoms, including corneal / conjunctival edema and red eye reflex. However, there was no difference in the cellular response of the anterior chamber. But in our study, the statistical population was human and anterior uveitis was treated. Similarly, to the above study, the results of the nano-micellar group DEX were significantly better than the conventional DEX group and all the studied variables in the nano-drug group had a faster process for treatment. In the above study, the difference between cell and fibrin changes in the anterior chamber was not statistically significant, but in our study, this variable also improved significantly faster in the nano-drug group. Of course, it is noteworthy that the statistical group of rabbits is not easy enough to examine the cell in the anterior chamber and may differ from our results in this regard. Also endotoxin induced in rabbit eyes caused pan-uveitis, but the patients we studied had only anterior uveitis. However, the concordance of the results of more effective treatment with the nano-micellar drug DEX in both studies necessitates more attention to nanotechnology in the production of topical ophthalmic drugs.

Study of Rafei et al., (2010) results showed that topical administration of these nanoparticles significantly reduced the symptoms of uveitis, and statistical analysis showed that the nanoparticle significantly reduced inflammation within 48 hours after endotoxin injection. In comparison with Rafei's study with ours, the better efficacy of micellar DEX nanoparticle compounds in intraocular inflammation is considered and the importance of our study compared to the above study is drug evaluation in comparison with the control group in humans while Rafei's study was tested on rabbit's eye. Another advantage of our study over the above study is the use of quantitative and more reliable variables.

In 2013, Mr. Moya and colleagues compared a combination of DEX and cyclodextrin (DexNP) with a high-concentration nano-carrier DEX to DEX alone (containing a benzalonium carrier). Their results showed that the maximum concentration of the drug in humor aqueous was 2 hours after drug administration, while in the combined form it was 136mic/ml and in the DEX form it was only 44mic/ml. The result of this study is better penetration and pharmacokinetics of DEX on the cyclodextrin nano drug carrier compared to our study, while in our study this effect was better and the clinical symptoms improved rapidly in nano-micellar DEX carrier.

5. CONCLUSION

The use of micellar DEX nanoparticles in different doses and concentrations depending on the site of action of the drug, which is the anterior segment or the drug wants to act in the posterior segment, and their replacement instead of topical DEX can be quite effective. Of course, the need for further studies and clinical trials at different levels can indicate the effectiveness of this drug in human societies. Therefore, more controlled studies are needed to understand and recognize the effects of DEX nanoparticles in the treatment of uveitis.

Acknowledgments

This article is extracted from final thesis of Dr. Alinejat Sharafi for his course of ophthalmology residency with registration number IORC-9708. This study was supported by Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Ethical approval

The study was accepted by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences. Written, informed consent was obtained from each patient. The study was enumerated in Iranian Registry of Clinical Trials (registration number, IRCT20190218042750N1).

Conflicts of interest

The authors declare that they have no conflict of interest.

Funding

This study has not received any external funding.

Data and materials availability

All data associated with this study are present in the paper.

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